"Gravitational Effects on Signal Transduction"

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The purpose of this study is to investigate the effect of microgravity on the signaling pathway triggered by the hematopoietic growth factor erythropoietin (Epo) and to determine whether this is associated with the anemia of space flight.

We chose to use a model cell culture system with which we have long and successful experience. These cells, designated Rauscher murine erythroleukemia, grow independently in suspension culture. When exposed to erythropoietin, they undergo a differentiation pathway remarkably similar to normal erythropoiesis. During this project period, we adapted these cells to growth in the NASA RWV. We employed the disposal chamber model produced by Synthecon, Inc. In a series of experiments, we first compared the growth rate of Rauscher cells under conditions of simulated microgravity with that of cells grown in standard tissue culture flasks. We observed that over a 3-4 day period, Rauscher cells in the RWV grew at approximately 50% the rate of those in standard flasks. Examination of the cells for viability using trypan blue staining demonstrated greater than 95% viable cells under both conditions of growth. Additionally, no increased apoptosis was observed in the RWV cells using the standard cell staining assay. From these studies we conclude that the simulated microgravity conditions of the RWV result in reduced proliferation of our erythroid cell model system.

We next began to study the effect of simulated microgravity on erythropoietin induced differentiation of these cells. In this series of experiments, cells were grown either in tissue culture flasks or in the RWV. At a specified time, recombinant human erythropoietin was added and the cells were allowed to continue to grow. At specified times thereafter over a four day period, small aliquots of cells were removed and scored for cell number and percent of hemoglobinized cells as a marker of Epo induced differentiation. In two separate experiments, we observed a marked inhibition of Epo induced differentiation in cells grown in the RWV. Cells grown in tissue culture dishes achieved 18-25% hemoglobinization after 72 hours. In marked contrast, RWV grown cells achieved only 9-13% hemoglobinization. Thus, simulated microgravity results in reduced Epo induced erythroid cell differentiation as well as inhibited

erythroid cell growth.

Most of these observations are consistent with a defect in erythropoietin's signal transduction as a causative of the anemia of space flight. In the next year, we plan to investigate the molecular aspects of erythropoietin signaling in the cells under conditions of simulated microgravity and compare them with signaling in cells grown in tissue culture flasks.